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14. ABSTRACT
Tinnitus is a major health problem among those currently and formerly in military service. This project hypothesizes that many of the clinically-significant, non-auditory aspects of the tinnitus condition involve two major brain networks: the cognitive control network (CCN) and the default mode network (DMN). Using fMRI, we are examining brain activation in subjects performing cognitive tasks that engage the CCN and DMN. One task is heavily reliant on working memory (N-back) and the other on selective attention (counting Stroop). Each task is conducted on auditory stimuli and, separately, on visual ones. A second version of the selective attention task includes emotional priming stimuli (fearful faces) so the effect of affect on CCN/DMN function can be assessed. Subjects in three groups are being compared: (1) control subjects with clinically-normal hearing thresholds and no tinnitus, (2) tinnitus subjects matched in hearing to the controls, (3) tinnitus subjects with bilateral high-frequency hearing loss. So far twenty-one subjects have been behaviorally tested and imaged. Preliminarily, the results support our hypothesis that people with tinnitus may exert greater cognitive effort in order to achieve the same level of outward performance as non-tinnitus controls on challenging cognitive tasks. The data are also beginning to suggest particular brain areas within the CCN, especially, that may warrant targeting for treatment and/or monitoring to quantify treatment efficacy.

15. SUBJECT TERMS
tinnitus, cognitive control network (CCN), default mode network (DMN), functional magnetic resonance imaging (fMRI)

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1. Introduction

The overall goal of this project is to test whether two major brain networks and their connections with auditory cortex play an important role in tinnitus. The networks are the cognitive control network (CCN) and default mode network (DMN). The specific aims are as follows:

Aim 1: During demanding cognitive tasks, test whether tinnitus subjects show greater engagement of the CCN and DMN than controls (i.e. physiological evidence of greater cognitive load).

fMRI activation will be measured during auditory and visual versions of two demanding tasks heavily reliant on working memory (N-back task) and selective attention (counting Stroop task). Control subjects will be compared to two groups of tinnitus subjects, both matched in age and sex to the controls and one matched in hearing threshold (clinically normal). A subset of subjects in each group will be matched in performance (accuracy and reaction time) so any dependence of activation on performance can be distinguished from that of tinnitus. Engagement of the CCN will be measured as increased image signal during task conditions relative to no task or reduced task load conditions ("positive" fMRI activation). Engagement of the DMN will be measured as the opposite i.e., reduced image signal during task conditions ("negative" activation).

Aim 2: Determine whether the reduced resting state functional connectivity between primary auditory cortex (PAC) and CCN/DMN in tinnitus subjects is reinstated
(a) during demanding tasks in the auditory domain, but not during tasks in the visual domain and,
(b) only when tinnitus is not perceived during the tasks.
For Aim 2a, fMRI data from Aim 1 will be used to assess PAC-CCN/DMN functional connectivity during task performance on auditory stimuli and, separately, on visual stimuli. For Aim 2b, following each scan, tinnitus subjects will report on their tinnitus during the tasks of that scan. These experiments will take an important step toward identifying ways to manipulate PAC – CCN/DMN connectivity and showing whether or not this connectivity is in fact crucial to the defining experience of tinnitus, the percept.

Aim 3: Test whether the influence of emotional priming on CCN and DMN function during a demanding cognitive task is greater in tinnitus subjects than controls.
During fMRI, subjects will perform the same selective attention task as in Aim 1 (counting Stroop, visual and auditory versions) but with the addition of a brief, visual priming stimulus before each trial. CCN and DMN engagement by the selective attention task, as well as functional connectivity within the CCN and DMN, will be compared between two types of primes, fearful and neutral faces, and further compared between controls and each of the two tinnitus groups.

Specific hypotheses tested by each aim:
(1) During attention-demanding tasks, there is an extra cognitive burden on tinnitus subjects that results in greater engagement of the CCN and DMN compared to non-

tinnitus controls.

(2) Functional connectivity between PAC and the CCN/DMN in tinnitus subjects will approach that of controls (a) during performance of demanding cognitive tasks performed in the auditory domain, but not during tasks in the visual domain and, (b) when tinnitus is not perceived during the tasks.

(3) The CCN and DMN are more susceptible to hijacking by the ventral affective network in tinnitus subjects than in non-tinnitus controls.

2. Keywords (and abbreviations)

- tinnitus
- cognitive control network (CCN)
- default mode network (DMN)
- primary auditory cortex (PAC)
- working memory
- selective attention
- emotional priming

3. Accomplishments

3.1 Activities in relation to Statement of Work

Activities to date have followed the SOW:

Development of experimental paradigm - During the first months of the project, the behavioral tasks to be used in fMRI were developed (N-back, Stroop, and an emotionally primed Stroop). Preparing the tasks involved recording and processing auditory stimuli and generation of bitmaps for visual stimuli (numbers in varying spatial patterns) to be presented to subjects during fMRI. The stimuli were then incorporated into programs that control the timing of auditory and visual stimulus presentation relative to the fMRI scans while also acquiring subject button-press responses needed to quantify task performance. Programming was performed using the software package Presentation (Neurobehavioral Systems).

Subject recruitment and testing - Over the past year, 33 subjects were recruited through ads in local and university newspapers, the MEEI website, and postings in local stores. Of these, 21 met the criteria for this study and were invited to participate in three testing sessions:

Behavioral testing session in which audiograms are obtained, loudness growth and discomfort level are measured, tinnitus pitch, loudness are determined as well as the minimum level of broadband noise needed to mask the tinnitus percept (minimum masking level). Subjects are also familiarized with the tasks they will perform during fMRI.

fMRI session 1 in which subjects perform a working memory task ("2-Back") and a simple detection task ("Detect 1's") based on (a) visual and (b) auditory stimuli. Resting state fMRI data are also acquired unless the subject has become too uncomfortable or tired, in which case resting-state measurements are deferred to session 2.

<u>fMRI session 2</u> in which subjects perform a selective attention task (Stroop) and a simple counting task based on (a) visual and (b) auditory stimuli.   Subjects also perform the auditory version of the tasks in the presence of emotional priming (brief presentation of fearful faces). Resting-state data are obtained if they weren't already in session 1.

Subjects recruited fall into three groups, as originally proposed:

(1) <u>NHcon</u> - control subjects without tinnitus and with clinically-normal hearing thresholds ($\leq$ 25 dB HL from 250 – 4000 Hz,$\leq$ 35 dB HL at 8000 Hz).

(2) <u>NHtin</u> – tinnitus subjects matched in hearing threshold to the NHcon group.

(3) <u>HFLtin</u> – tinnitus subjects with bilateral high-frequency loss.

Thus far, all 21 subjects have completed the behavioral testing session and fMRI session 1. 17 of these subjects have also completed fMRI session 2. The distribution of participants across subject groups is summarized in Table 1. Mean audiograms for each group are given in Figure 1. All subjects are men, so the groups are automatically sex-matched. Subject ages range from 35 to 59. Mean ages for each group are 44 yrs (NHcon, NHtin) and 50 yrs (HFLtin). Future recruitment will attempt to narrow the age gap between the NH groups and the HFLtin group so as to achieve the study goal of sex and age-matched groups.

Table 1: Summary of completed sessions in relation to project target.

	fMRI Sessions* Completed in Year 1				
	Session 1 N-Back	Session 2 Stroop**			
NH controls	9	8	* Resting state data are obtained during either session 1 or 2.		
NH tinnitus	6	5	**Includes Stroop with emotional priming for Aim 3.		
HFL tinnitus	6	4			
Total Completed	21	17			
Project Target	60	60			

Figure 1: Mean of left and of right audiograms for subjects in each group: NH con (black, 9 subjects), NH tin (red, 6 subjects), HFL tins (green, 6 subjects). Error bars have been omitted for clarity.
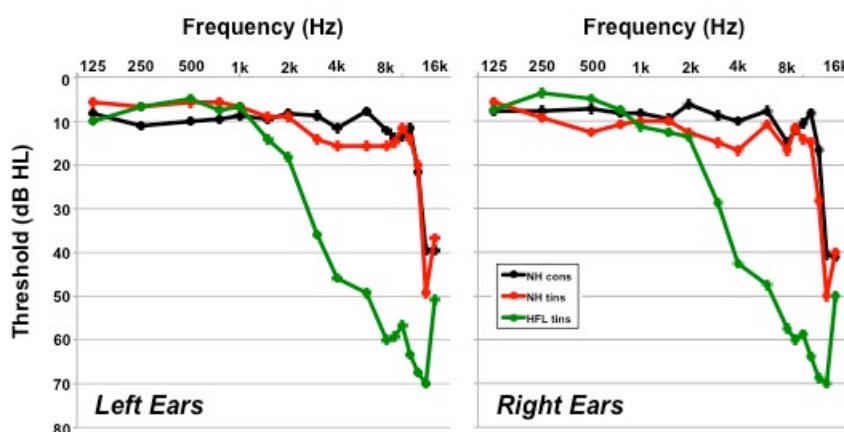
Data analysis – Using a combination of home-grown software and Statistical Parametric Mapping (SPM8; a freely available fMRI analysis package), fMRI and structural imaging data from each session were aligned to a standard brain atlas,

corrected for subject motion and inspected for artifacts. Activation maps were created for various contrasts, including the most basic contrasts described in section 3.2 below.

3.2 Preliminary Results

Behavioral data - Most subjects in all groups were able to perform the challenging tasks during both fMRI sessions. Exceptions were two control subjects who performed far more poorly than other subjects. These outliers have been omitted in inter-group comparisons of performance and imaging data below.

Analysis of the behavioral data taken during imaging showed little difference between subject groups in either response time or accuracy (calculated as   1 – (missed targets + false alarms)/total number of targets). The similarity can be seen in Figure 2 for the 2-back task of session 1 (left panels of A and B, respectively). During the detect 1's task of session 1, subjects performed with perfect or near-perfect accuracy (i.e., accuracy = 1; not shown). The similarity in mean response time across subject groups can be seen on the right in panels A and B for both the detect 1's and 2-back tasks.

Figure 2: Accuracy and response time (RT) data from fMRI session 1 during which subjects detected 1's in some blocks and performed a 2-back task in others. (A) Data for visual version of the tasks. (B) Data for auditory version. Mean across subjects of each group: NHcon (gray bars), NHtin (red), HFLtin (green). Error bars indicate +/- one SEM.
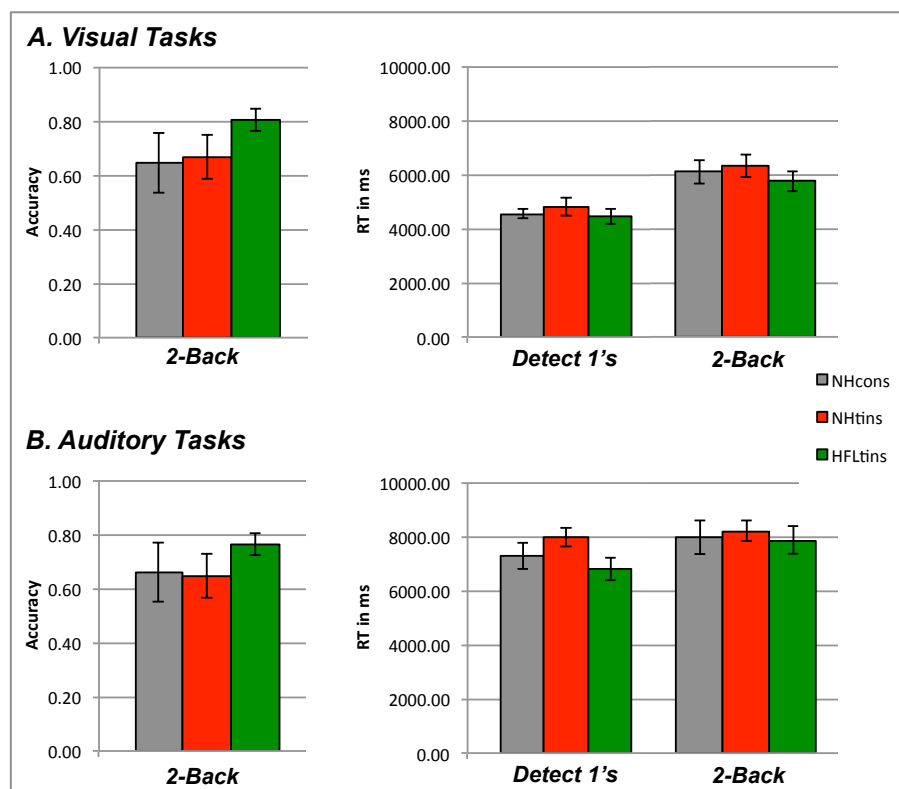
Cognitive control network – Figure 3 shows maps of fMRI activation based on the session 1 data of all subjects. Specifically, image signal during the detect 1's and 2-back task conditions was contrasted with image signal during intervening periods of fixation (no task). The resulting contrast maps were then pooled across subjects to identify brain regions showing significant activity increases (i.e., image signal increases) during task conditions (colored regions in Figure 3). Regions showing a significant

activity increase were then targeted in a secondary analysis to determine whether the activity increases differed between tinnitus subjects (NHtin and HFLtin) and controls (NHcon). Several areas showed significant differences, which in all cases corresponded to greater task-related activity increases in the tinnitus groups. When the tasks performed on auditory stimuli, anterior insular cortex and left auditory cortex showed greater increases in activity in the tinnitus groups compared to controls. When the tasks were performed on visual stimuli, right anterior insula and left orbital-frontal cortex showed greater increases in the tinnitus groups. While other parts of the cognitive control network were activated during the tasks (dorsolateral prefrontal cortex, supplementary motor cortex (SMA)/pre-SMA), they did not show differences in activity between subject groups.
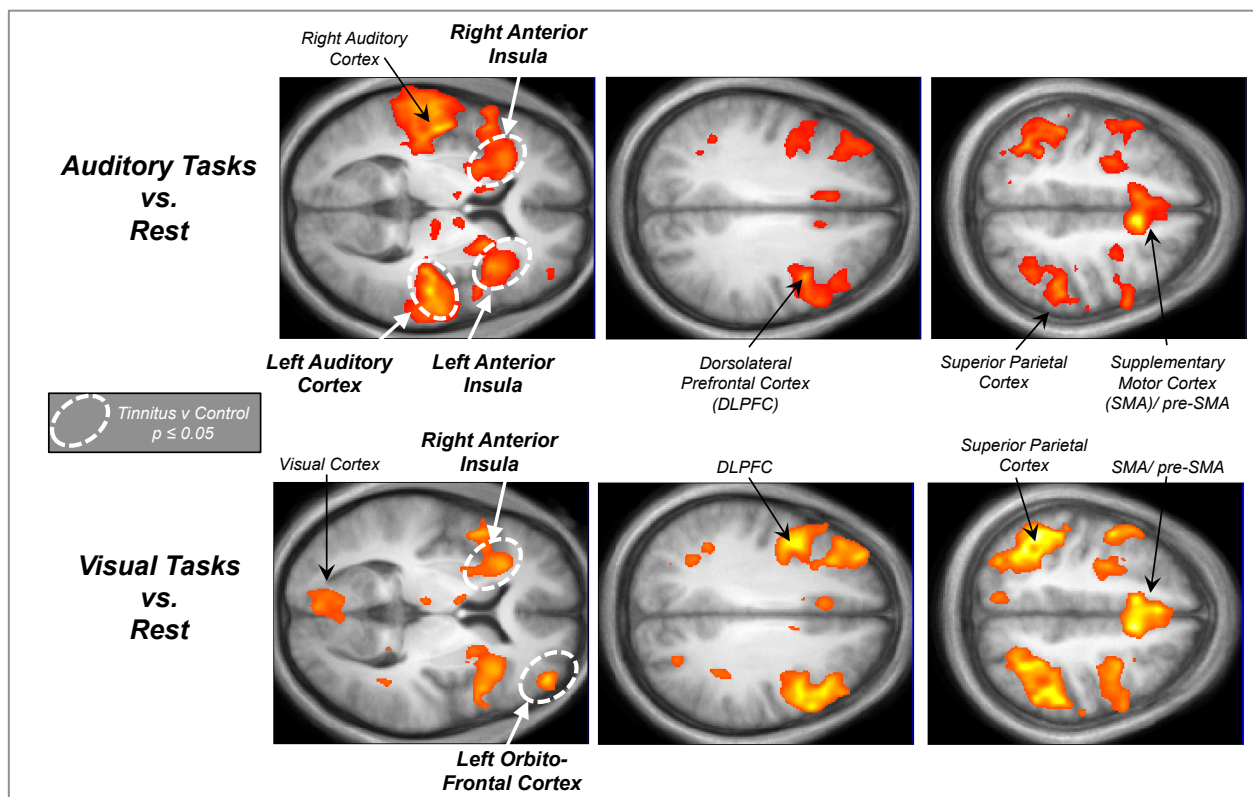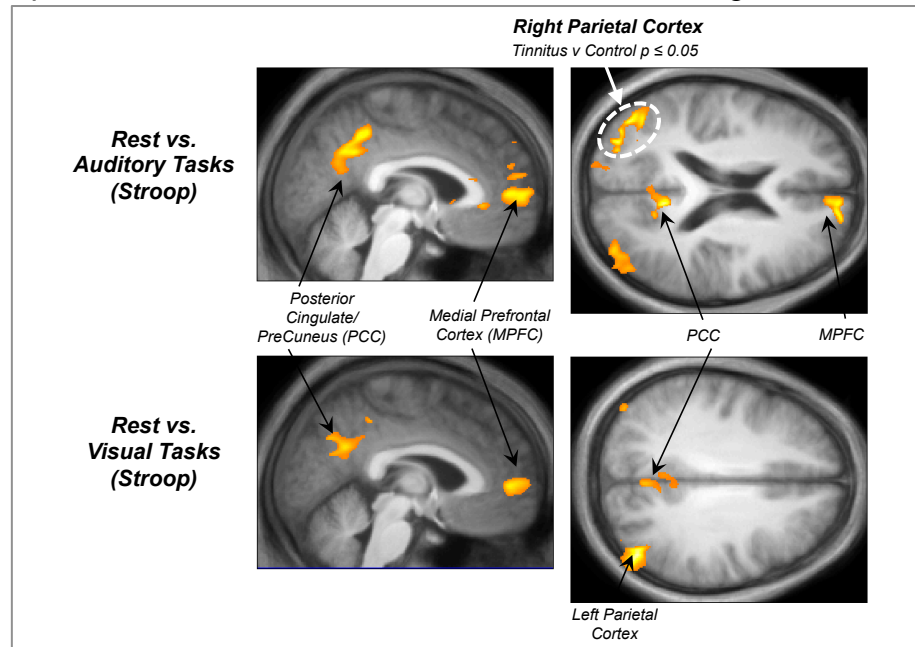

Figure 3: Cognitive control network. fMRI session 1 data. Axial brain slices showing activation maps based on (A) auditory and (B) visual task conditions (detect 1's and 2-back) contrasted with no-task periods. Contrasts determined for individual subjects were pooled across subjects/groups in a second-level analysis. Color indicates brain areas of significant activity increase ($p < 0.0001$, uncorrected) during task performance. Increasing significance is coded from red to yellow. The activation maps are superimposed on a mean of structural scans (average over the 21 subjects contributing to the activation maps). Dashed white ovals and boldface labeling indicate brain areas showing a significant difference in contrast magnitude between the tinnitus groups (NHtin, HFLtin) and the control group (NHcon).

Default mode network – Figure 4 shows maps of fMRI activation based on the session 2 data of all subjects. In this case, image signal during no-task periods has been contrasted with the task conditions and the contrast maps pooled across subjects.

Color indicates brain regions where activity (i.e., image signal) was greater when no task was performed (i.e., regions comprising the default mode network). Here again, regions were examined for significant differences between the tinnitus groups and the control group. One, right parietal cortex, showed a difference. The other regions did not.

Figure 4: Default mode network. fMRI session 2 data. Sagittal (left) and axial (right) brain slices showing activation maps based on (A) auditory and (B) visual versions of the tasks in session 2. See Figure 3 caption.

Right Parietal Cortex
Tinnitus v Control p ≤ 0.05

Rest vs. Auditory Tasks (Stroop)

Posterior Cingulate/ PreCuneus (PCC)

Medial Prefrontal Cortex (MPFC)

PCC

MPFC

Rest vs. Visual Tasks (Stroop)

Left Parietal Cortex

3.3 Implication of results for the project hypotheses and future plans

The data obtained so far provide preliminary support for our first hypothesis:
(1) During attention-demanding tasks, there is an extra cognitive burden on tinnitus subjects that results in greater engagement of the CCN and DMN compared to non-tinnitus controls (Figures 3, 4). - The fMRI data that will be obtained in year 2 and early year 3 will enable a stronger test of this hypothesis.

A next step will involve analyzing both already-obtained and to-be-obtained data to test our second hypothesis:
(2) Functional connectivity between PAC and the CCN/DMN in tinnitus subjects will approach that of controls (a) during performance of demanding cognitive tasks performed in the auditory domain, but not during tasks in the visual domain and,
(b) when tinnitus is not perceived during the tasks. – The research assistant hired in the early months of this project is being trained to do fMRI data analysis as illustrated in this report. She will next be trained in the methods of functional connectivity, so she can assist with this highly time-consuming analysis of the fMRI data needed to test this second hypothesis.

Additional data will be necessary for even a preliminary test of our third hypothesis:
(3) The CCN and DMN are more susceptible to hijacking by the ventral affective network in tinnitus subjects than in non-tinnitus controls. - Data bearing on this hypothesis have been, and will continue to be, collected (see Table 1).

4. Impact

This project is important for multiple reasons, including the fact that it stands to implicate particular brain networks and/or synergies between networks in the aspects of tinnitus that make tinnitus a clinical problem. Any well-controlled, quantitative physiological study of tinnitus has the potential for yielding a tinnitus biomarker. The proposed study is not an exception. Such a measure based on the present study paradigm holds special appeal because it could be used to objectively test the efficacy of therapies directed at improving the cognitive management of tinnitus, therapies based on attention tasks, or mind-body therapies such as meditation; in other words, therapies targeting networks that transform the tinnitus percept from benign to problematic.

5. Changes/ Problems

There have been no major changes to this project. The following minor changes were made, none of which compromises the project goals:

(1) Because subjects' time in the scanner was proving too long: (a) resting state fMRI data is being obtained during one instead of both of the fMRI sessions, and (b) effects of emotional priming (Aim 3) on auditory (but not visual) task performance is being examined.

(2) Subjects have so far been recruited via flyers posted widely throughout Boston and surrounding communities instead of via subway ads (as originally proposed) in order to diversify the demographics of people inquiring about participation. The flyering approach does, however, have a down-side in being labor intensive and low in yield, so we will likely use subway ads for recruitment in year 2. Some of the money originally allocated to advertising in year 1 was used to replace a computer that unexpectedly failed. The computer is essential as it is used for downloading image data from the scanner and for some aspects of data analysis.

(3) Late last spring, the Martinos Imaging Center reduced the hourly rate for use of the scanner from $847 to $588. This was fortunate because, for various reasons, it has proven quite difficult to limit the fMRI sessions to one hour apiece. We were in danger of running out of money for imaging. Even though the hourly rate of scanning has been reduced, it is essential that our total imaging budget for years 2 and 3 remain unchanged as it will allow us to conduct 1.5 hour fMRI sessions, as needed, instead of 1-hour sessions.

6. Products

None.

7. Participants & Other Collaborating Organizations
The human subject testing for this project has been approved by HRPO.

8. Special Reporting Requirements
None.

9. Appendices
None.